Affinity of Hemoglobin for Oxygen and Prooxidant-Antioxidant Equilibrium after Administration of Lipopolysaccharide during Correction of the L-Arginine—NO Pathway

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 6, pp. 616-619, June, 1999
Original article submitted April 16, 1998

Effects of correction of the L-arginine—NO pathway on the fever reaction, oxygen transport function of the blood, and prooxidant-antioxidant equilibrium in rats injected intramuscularly with lipopolysaccharide were studied. pH, Pco_2, Po_2, and the index of hemoglobin oxygen affinity (p50) were measured in mixed venous blood. Levels of Schiff bases, α-tocopherol, and catalase activity were determined in erythrocytes and in the liver, kidneys, and heart. NO synthase inhibitor attenuated the fever reaction and decreased p50 to 28.89±0.83 mm Hg (in rats administered with lipopolysaccharide, p50 was 34.21±1.63 mm Hg). The increase in the content of Schiff bases and the exhaustion of the antioxidant system in erythrocytes and tissues were less pronounced in rats injected with the NO synthase inhibitor than in animals receiving lipopolysaccharide only. Various parameters of the prooxidant-antioxidant equilibrium correlated with p50. Thus, hemoglobin oxygen affinity and NO are important factors involved in the maintenance of the prooxidant-antioxidant equilibrium in the body.

Key Words: *hemoglobin oxygen affinity; lipid peroxidation; lipopolysaccharide; nitric oxide*

In most cases, the production of prooxidants in tissues is balanced by intra- and extracellular antioxidants. Thus, the optimal level of the prooxidant-antioxidant equilibrium is established [6]. Studies of the fever reaction and the directed modification of hemoglobin oxygen affinity (HOA) in fever [3,13] showed that the oxygen transport system is involved in the maintenance of this equilibrium [2].

Various biological effects of nitric oxide (NO) are now extensively studied. NO is an unique molecule that acts as a physiological messenger and sometimes as a cytotoxic effector molecule [12]. NO is formed from L-arginine under the effect of NO synthase in the presence of NADPH, calmodulin, and other cofactors. NO plays an important role in the regulation of vascular tone. At the same time, NO binds to hemoglobin with the formation of nitrosohemoglobin and interacts with superoxide anion yielding an extremely potent oxidant peroxynitrite [7]. On the other hand, O_2 is an important regulator of NO synthase activity in hypoxic tissues [11. Here we studied the interrelation between HOA and parameters of the prooxidant-antioxidant equilibrium during correction of the L-arginine-NO pathway in fever.

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MATERIALS AND METHODS

Experiments were performed on male rats weighing 200-280 g. The animals were kept in a vivarium at 20°C and divided into 6 groups. Group 1 rats (control, n=6) were intraperitoneally injected with isotonic NaCl. Group 2 rats (n=7) received intramuscular injection of 0.1 mg/kg *Salmonella typhi* lipopolysaccharide (LPS, N. F. Gamaleya Institute of Epidemiology and

0007-4888/99/0006-0558$22.00

Microbiology, Russian Academy of Medical Sciences). Group 3 rats (n=11) were injected intraperitoneally with 30 mg/kg nitroglycerin (Isis-Chemie). Group 4 rats (n=6) received LPS and nitroglycerin. Group 5 rats (n=8) received daily intraperitoneal injections of 25 mg/kg N^G-nitro-L-arginine methyl ester (L-NAME, Sigma) for 3 days. Group 6 rats (n=7) received LPS and L-NAME. The fever reaction was analyzed by the rise of rectal temperature. Blood from the right atrium and tissue samples (liver, kidneys, and heart) were taken 120 min after administration of pharmacological agents.

The HOA index (Po_2 corresponding to 50% oxygen saturation of hemoglobin) was measured by a modified mixing method [1] at 37°C, pH 7.4, and Pco_2=40 mm Hg ($p50_S$); p50 corresponding to real pH, Pco_2, and temperature ($p50_R$) was calculated by formulas [9]. Po_2, Pco_2, and pH in blood samples (0.13 ml) were measured on an ABL-330 gas analyzer (Radiometer) at 37°C. The actual base excess (ABE) and plasma concentrations of total CO_2 (TCO_2) and hydrocarbonates (HCO_3^-) were determined by formulas [9] and Siggaard-Andersen nomographs.

The content of Schiff bases (SB) was determined by the intensity of fluorescence of the chloroform extract in an F-4010 spectrofluorometer (Hitachi) at excitation and emission wavelengths of 344 nm and 440 nm, respectively [8]. Catalase activity was estimated by the amount of H_2O_2 interacting with molybdenum salts with the formation of stable stained complexes. The concentration of complexes was measured on an SF-46 spectrophotometer at 410 nm [5]. The content of α-tocopherol was determined by the intensity of fluorescence of heptane extract on an F-4010 spectrofluorometer (Hitachi) at excitation wavelength of 292 nm and emission wavelength of 325 nm [8].

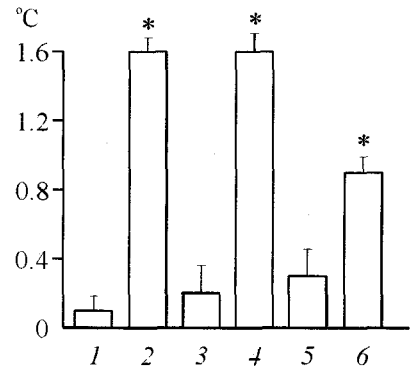

Fig. 1. Changes of rectal temperature in rats receiving 0.9% NaCl (*1*), LPS (*2*), nitroglycerin (*3*), LPS+nitroglycerin (*4*), L-NAME (*5*), and LPS+L-NAME (*6*). Here and in Fig. 3: *p<0.05 compared with the control.

The data were processed by the multiple correlation-regression analysis (Statgraphics software).

RESULTS

Administration of LPS during the correction of the L-arginine—NO pathway changed parameters of the blood oxygen transport function, lipid peroxidation (LPO), antioxidant system (AOS), and temperature homeostasis in rats. However, these changes varied in various animal groups (Fig. 1). In group 6 animals receiving LPS before L-NAME, the temperature rise was minimum. Nitroglycerin and L-NAME (groups 3 and 5, respectively) had practically no effect on rectal temperature.

LPS induced metabolic acidosis (Table 1) and decreased the oxygen supply. Similar changes were observed after administration of LPS against the background of nitroglycerin (group 4). Injection of L-NAME

TABLE 1. Parameters of Blood Oxygen Transport Function in Rats Intramuscularly Injected with LPS during Correction of L-Arginine—NO Pathway ($M\pm m$)

Index	Control	LPS	Nitroglycerin	LPS+nitroglycerin	L-NAME	LPS+L-NAME
pH_S, units	7.282±0.006	7.220±0.013*	7.282±0.009	7.247±0.007*	7.295±0.08	7.276±0.009
pH_R, units	7.274±0.006	7.191±0.013*	7.270±0.012	7.217±0.007*	7.293±0.012	7.252±0.006
Pco_{2S}, mm Hg	50.33±1.84	51.89±1.47	50.56±0.64	50.08±1.20	50.70±1.22	49.48±1.31
Pco_{2R}, mm Hg	51.45±1.87	56.53±1.59	52.38±0.98	54.85±1.49	51.36±1.41	53.06±1.46
Po_{2S}, mm Hg	28.3±1.21	23.71±1.32*	26.35±1.90	24.33±0.68	26.78±0.68	25.78±1.40
Po_{2R}, mm Hg	29.37±1.43	27.28±1.55	27.90±2.10	28.20±0.71*	27.08±1.01	27.76±1.58
$p50_S$, mm Hg	33.7±0.413	37.68±0.41*	37.52±1.25*	36.13±0.54*	35.69±0.59*	30.81±0.95*
$p50_R$, mm Hg	30.06±0.48	34.24±1.63*	33.98±1.21*	34.06±0.80*	31.75±0.87*	28.89±0.83
ABE, mmol/liter	-2.97±0.93	-6.04±0.73*	-2.64±0.85	-4.65±0.81	-2.17±1.13	-2.96±0.87
TCO_2, mmol/liter	24.38±0.87	21.86±0.72*	23.73±1.06	22.15±1.41	23.63±1.55	24.54±1.28
HCO_3^-, mol/liter	23.32±1.03	20.27±0.71*	22.57±1.04	21.12±1.27	23.14±1.17	23.37±1.24

Note. Here and in Table 2: *p<0.05 compared with control.

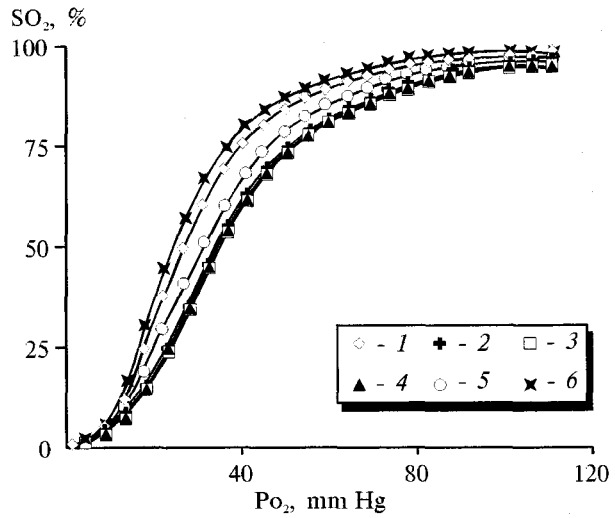

Fig. 2. Oxygen dissociation curves at actual values of pH, Pco_2, and temperature in rats receiving 0.9% NaCl (*1*), LPS (*2*), nitroglycerin (*3*), LPS+nitroglycerin (*4*), L-NAME (*5*), and LPS+L-NAME (*6*). Ordinate: degree of oxygen saturation of hemoglobin.

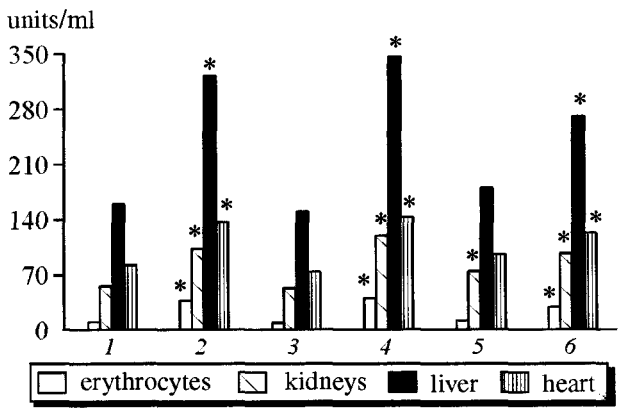

Fig. 3. Contents of Schiff bases in erythrocytes and tissues of rats receiving 0.9% NaCl (*1*), LPS (*2*), nitroglycerin (*3*), LPS+nitroglycerin (*4*), L-NAME (*5*), and LPS+L-NAME (*6*).

before LPS tended to restore these indexes. Nitroglycerin and L-NAME had no considerable effects on oxygen transport functions of the blood.

A leftward shift of the oxyhemoglobin dissociation curve (ODC) is reflected by changes in $p50_R$ (Fig. 2). Values of $p50_S$ and $p50_R$ in rats administered with LPS or nitroglycerin+LPS were similar, while nitroglycerin increased $p50_S$.

The intensity of LPO evaluated by the content of SB considerably increased in group 2 animals treated with LPS and in group 4 rats receiving LPS+nitroglycerin (Fig. 3). The inhibition of NO synthase produced a less pronounced effect. LPS reduced AOS indexes in all rats (Table 2). Catalase activity and the content of α-tocopherol in erythrocytes, kidney, liver, and heart decreased after administration of LPS or LPS+nitroglycerin. The inhibition of NO synthase induced protective effects. Parameters of LPO and AOS in the majority of tissues in rats administered with nitroglycerin and L-NAME (groups 3 and 5, respectively) did not differ from control values.

The ratio between various parameters was determined by the multiple correlation analysis; pair correlation coefficients were estimated. Moderate linear correlations were revealed between $p50_R$ and SB (r=0.66÷0.71) and catalase activity and the content of α-tocopherol (r=-0.43÷0.82). These data suggest the interrelation between HOA and free-radical oxidation indexes under conditions of correction of the L-arginine—NO pathway.

A shift of the ODC correlates with free-radical oxidation parameters in fever [3,13] and, probably, represents a physiological mechanism involved in the maintenance of the prooxidant-antioxidant equilibrium in the body. Various endotoxins induce the expression of inducible NO synthase. This leads to the formation of large amounts of NO that interacts with O_2^- with

TABLE 2. Parameters of Antioxidant Defense in Rats Intramuscularly Injected with LPS during Correction of L-Arginine-NO Pathway (*M±m*)

Index	Control	LPS	Nitroglycerin	LPS+nitro-glycerin	L-NAME	LPS+L-NAME
Catalase						
erythrocytes	579.99±10.39	226.96±4.32*	513.15±15.52	200.03±15.19*	548.02±12.80	257.89±25.58*
kidneys	216.32±2.82	141.61±3.44*	192.78±7.09	84.28±23.57*	217.94±16.4	135.99±12.13*
liver	406.24±9.85	314.05±5.38*	375.4±10.48	149.96±17.85*	395.55±11.37	268.42±39.36*
heart	36.52±0.22	21.74±0.38*	34.35±0.61	13.07±5.42*	35.56±0.42	25.04±2.75*
α-Tocopherol						
erythrocytes	36.86±1.52	27.29±0.33*	38.54±5.23	25.43±1.10*	34.97±1.68	27.51±0.76*
kidneys	80.99±1.03	68.16±0.34*	76.75±1.45	64.97±1.86*	78.94±2.19	69.16±1.07*
liver	78.45±2.1	65.42±0.40*	75.28±1.66	62.07±1.82*	76.98±2.33	65.93±1.18*
heart	80.61±2.20	62.50±0.63*	74.74±2.18	57.17±2.77*	78.74±2.42	62.79±1.35*

the formation of peroxynitrite [7,12]. The observed decrease in the intensity of fever reaction and reduced accumulation of LPO products are obviously due to inhibition of inducible NO synthase and attenuation of its negative effects associated with NO overproduction. The inhibition of NO synthase attenuates fever response to LPS and is accompanied by a leftward shift of the ODC and less pronounced activation of free-radical oxidation.

Our experiments and previous studies show that the antioxidant defense is not restricted by the cellular level only. The concept of free-radical oxidation as a function of oxygen concentration in cells suggests that this antioxidant defense is more complex. The optimum oxygen concentration and the intensity of LPO depend on the oxygen transport system and its individual components. Sometimes, various antioxidant mechanisms do not prevent harm but only combat their consequences. Therefore, the decrease in the intracellular concentration of oxygen could solve the problem of oxygen danger [4]. HOA determining oxygen diffusion into tissues and tissue Po_2 plays a significant role in the complex AOS [2].

Our findings show a close interrelation between HOA and free-radical oxidation parameters during inhibition of NO synthesis under conditions of intramuscular administration of LPS. These data indicate that changes in oxygen-binding capacity of hemoglobin is an important physiological mechanism maintaining the prooxidant-antioxidant equilibrium.

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